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Polylactic acid films implantation into the anterior chamber of eye *in vivo*

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Abstract. The purpose of this research is the study of the influence of thin polylactic acid films implantation on the corneal morphology *in vivo* experiment. Studies were performed on 8 pubescent female Sylvilagus bachmani rabbits. Polylactic acid films were implanted into the anterior chamber of one animal eye. The feedstock for the films was obtained by dissolving polylactic acid in the chloroform. Before the implantation PLA films were sterilized. The overall duration of the *in vivo* experiment comprised 21 days. Such methods as visual check, photographic registration, non-contact ocular tonometry and optical coherent tomography of cornea were also used in course of the experiment. Sampling was performed on day 21 after the start of the experiment for morphology studying. Results showed that the implantation of the PLA films into the anterior chamber of eye does not induce an inflammatory reaction and does not increase an intraocular pressure. The study showed the possibility of PLA films using as a corneal implant.

1. Introduction

Polylactic acid (PLA) is a biodegradable aliphatic non-toxic and eco-friendly polyester and is widely used as medical implants in the form of anchors, screws, plates, pins, rods, and as a mesh [1 - 4]. A comprehensive literature search reveals the applications of PLA and its polymeric composites in medical fields such as: orthopedics, drug carriers, facial fracture repair, tissue engineering, antimicrobial agents, antitumor, ureteral stents, biomaterials, miscellaneous applications [1 - 4]. Of particular interest is the use of PLA as a corneal implant for the bullous keratopathy treatment. The bullous keratopathy is the severe cornea disease and occupies one of the leading positions among the causes of corneal weak vision. Primary and secondary processes of the degenerative and infectious diseases of the cornea play a significant role in the development of bullous keratopathy which lead to the death of endotheliocytes and dysfunction of the corneal endothelial layer [5]. This contributes to the development of corneal edema, reduced vision, the occurrence of recurrent erosions and pronounced pain symptom.

One of the directions in the treatment of this disease is the use of stem cells [5]. However, cultivation of stem cells and their landing on the inner surface of the cornea in order to replace the endothelial defect without using a substrate is a big surgical problem. There is a high probability of cell loss during surgical procedures. Using of scaffolds based on biodegradable thin films with controlled solubility as a temporary material for stem cells may be an alternative to existing insoluble polymers.

The purpose of this research is the study of the influence of thin polylactic acid films implantation on the corneal morphology *in vivo* experiment.

2. Material and methods

2.1. Polylactic acid films
The feedstock for the films was obtained by dissolving polylactic acid (PURASORB® PL 10, Netherlands) in the chloroform ($CHCl_3$). 1% solution was poured into Petri dishes (10 g) which were placed in a fume hood until complete evaporation of $CHCl_3$ and the formation of polylactic acid films.

2.2. Sterilization
The steam sterilization was carried out in a GPA-10 PZ (Russia) autoclave, operating at the temperature of 120°C - 121°C and pressure of 0.11 MPa for 20 min.

2.3. The surface topography of polylactic acid films
The surface topography was studied on the multipurpose correlator of optical, spectral and topographical surface objects properties "Centaur HR" (Russia). The surface roughness was estimated using the Gwyddion software. SEM of polylactic acid films was obtained by using Hitachi S3400N Type II microscope (Japan).

2.4. The wetting angle of polylactic acid films
The wetting angle for purified water and glycerol were calculated using the sessile drop technique with the room temperature of 25±2°C, the "KRÜSS EasyDrop DSA 20" device (Germany) and the special software, with the measurement accuracy of ± 0.1°. The surface energy was calculated using Owens, Wendt, Rabel and Kaelble method.

2.5. Animals and treatments
8 pubescent female Sylvilagus bachmani rabbits (SSMU, Tomsk, Russia) weighing 2.0-2.5 kg were used. All animals were healthy and free of ocular diseases. All procedures were approved by the Siberian Medical State University Life Science Ethical Review Committee (protocol № 3898 from November 24th, 2014).

Polylactic acid films were implanted into the anterior chamber of one animal eye. All animals were instilled Tobramycin Drops (6 times per day), 0.1% Diclofenac Sodium Ophthalmic Solution (3 times per day) and 0.05% Vitabact (4 times per day) in the postoperative period.

The overall duration of the experiment comprised 21 days. Such methods as visual check, photographic registration, non-contact ocular tonometry and optical coherent tomography (OCT) of cornea were also used in course of the experiment. Sampling was performed on day 21 after the start of the experiment for morphology studying.

2.6. Optical Coherence Tomography (OCT) of cornea
Optical coherent tomography of cornea was carried out on days 7, 14, and 21 after the start of the experiment on Cirrus HD-OCT 5000 (Germany).

2.7. Non-Contact Ocular tonometry
Non-Contact Ocular tonometry was done using Non-Contact Tonometer Huvitz HNT-7000 (South Korea).

2.8. Histological processing specifics
The enucleated animal eyeballs were put into the Carnoy's fixative solution for 2 hours, in course of the preparation for the light microscopy. Sections of tissues were stained with hematoxylin and eosin, according to the method of Van-Gizona. Light microscopy of the slides was conducted with 200x and 400x zoom using LOMO Biolam AU-12 (Russia) microscope. The microscope has an integrated digital camera ICC50 with the USB interface.

2.9. Electron microscopy of tissues
The study of the cytology structure was performed by transmission electron microscopy. The tissue was fixed in a 2.5% solution of glutaraldehyde for 2 hours at a temperature of 4°C. Then it was washed twice

with cacodylate buffer, was fixed in 1% solution of four osmium oxides (in 0.1 M cacodylate buffer) for 2 hours, and washed with cacodylate buffer again. Ultrathin tissue sections of 60–100 nm thick were prepared on an "Ultrotome III" ultratome (LKB, Sweden). The resulting sections were examined in a JEM-100 CXII electron microscope (JEOL, Japan) with an aperture diaphragm of 25–30 μm at an accelerating voltage of 80 kV.

2.10. Statistical processing of the research results

Statistical package IBM SPSS Statistics 20 was used for the statistical processing of the obtained results. A Student's t-test for unpaired values was used to analyze statistical significance between four groups. The Kruskal–Wallis test was used for the quantitative data. The Fisher's exact test was used for the nominal data. The dynamics analysis was conducted using the Wilcoxon signed rank test, which is used when comparing the mean value of two paired tests. Values of $p < 0.05$ were considered to be statistically significant.

3. Results

3.1. The surface topography of PLA films

The study of the structure and morphology of polylactic acid films showed that its roughness and topography depended on the side of the surface of the material: the outer side (contact with the atmosphere) had a more embossed surface as opposed to a smoother inner surface (contact with the Petri dish) (Figure 2, 3).

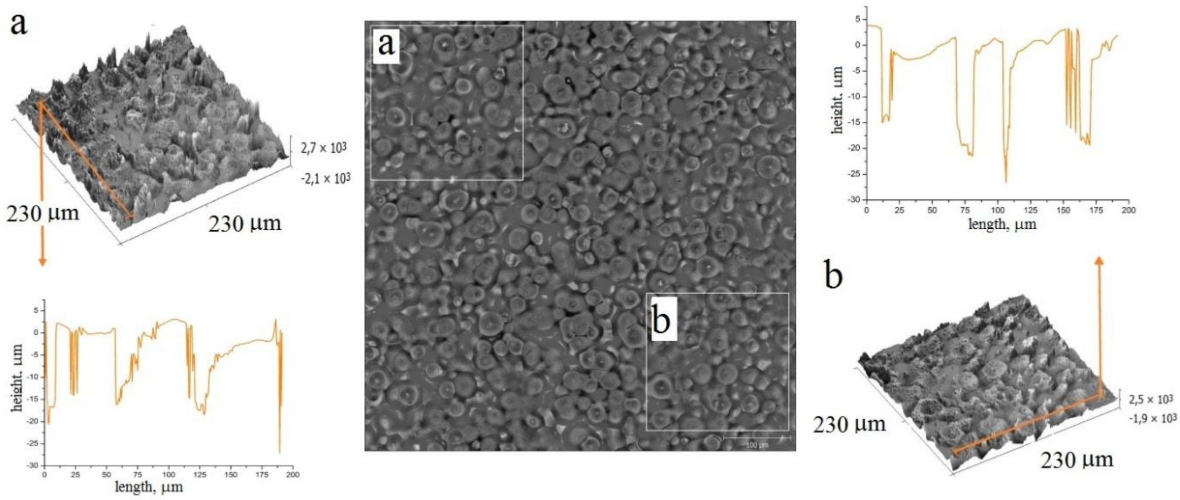

Figure 1. The surface topography of the PLA outer side.

Thus, the inner side of the material is formed smoother.

The surface roughness analysis showed that R_a of PLA films (inner) surface was 0.01±0.003 μm, R_q was 0.014 μm, R_{sk} was -0.001. PLA films (outer) surface had R_a = 0.17±0.06 μm, R_q=0.4 μm, R_{sk}=-1.0.

The sterilization increased the inner surface roughness of PLA films by 5 times. R_a of PLA films (inner) surface was 0.05±0.01 μm, R_q was 0.09 μm, R_{sk} was -0.66.

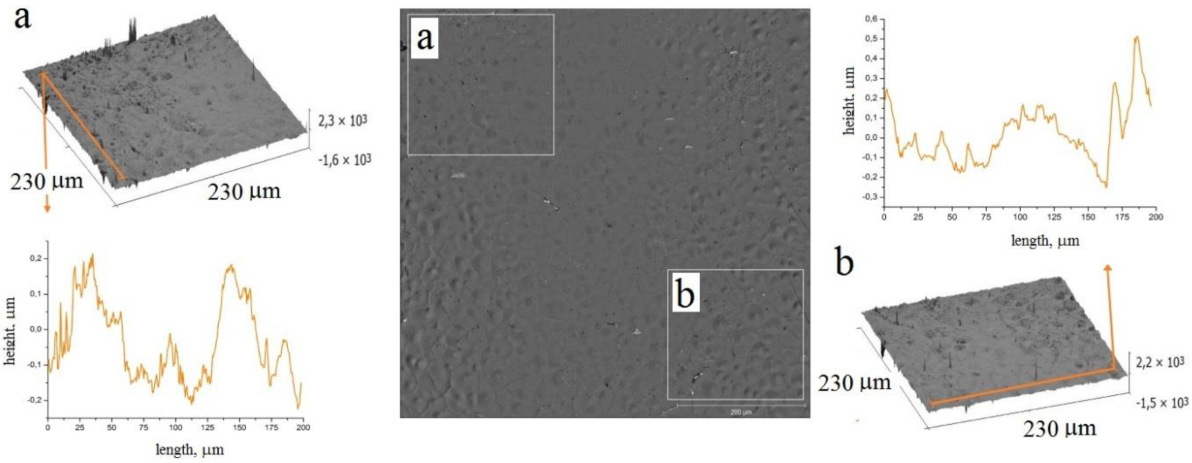

Figure 2. The surface topography of the PLA inner side.

3.2. The wetting angle of PLA films

The wettability of the surface analysis showed that the PLA inner side had a wetting angle of water of 80.5°±1.2. The PLA film properties are close to hydrophobic. The surface energy of the films varies within (26 ÷ 27) mJ/m^2. The polarity of PLA film (inner side) was 0.36. The PLA outer side had 78.5°±1.3. The polarity of PLA film (outer side) was 0.37.

The sterilization increased the PLA wettability and decreased the wetting angle of water. The wetting angle of PLA films after sterilization was 64.5°±2.3.

3.3. Visual check and Ocular tonometry

The implantation of the PLA film did not cause an inflammatory reaction and did not increase an intraocular pressure.

3.4. OCT of cornea

According to OCT the cornea had a normal thickness (430-450 μm) (Figure 3). There was observed a free position of the PLA film without contact with the iris and with the anterior chamber angle.

3.5. Histology and electron microscopy results

The following histological results were obtained. The anterior epithelium was represented by 4-5 layers of squamous epithelium with normochromic nuclei. Bowman's membrane was unchanged and visualized as a homogeneous eosinophilic strip. Collagen fibers were located compactly. In some places collagen fibers had increased twisted stroke (Figure 3a). Descemet's membrane was visualized throughout. The endothelial layer was represented by a single layer of cells. In some places proliferation of endothelial cells in the form of process cells was observed.

Electron microscopy showed that fibroblast had the normal structure (big oval shaped nucleus with dimensionally pronounced chromatins, normal structure mitochondrion, not extended tanks of rough endoplasmic reticulum) (Figure 4a). In most cases collagen fibers were located compactly. In some places collagen fibers had increased twisted stroke (Figure 4b).

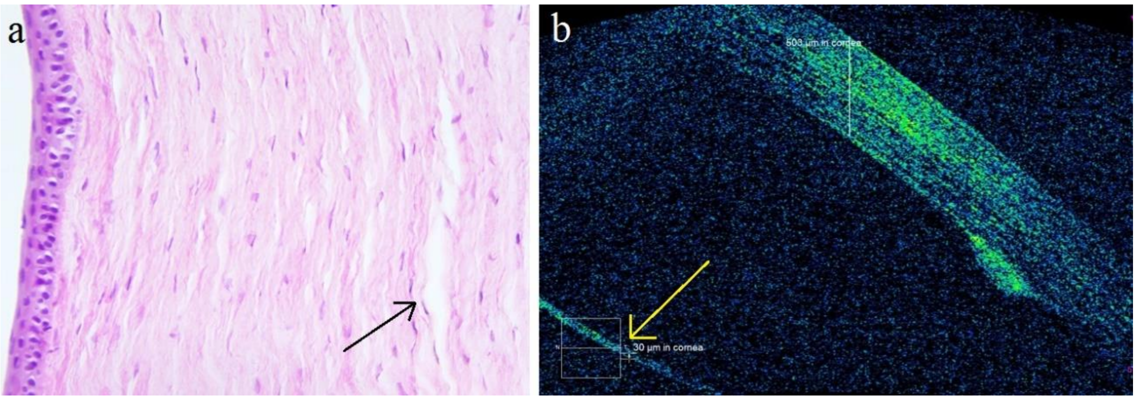

Figure 3. Histology tissue cross-section (a) and OCT of cornea (b) on day 21 after the start of the experiment *in vivo*. The black arrow shows extra twisted move of collagen fibers. The yellow arrow shows the PLA film into the eye anterior chamber. The magnification (a) is x200. Hematoxylin and eosin stain (a).

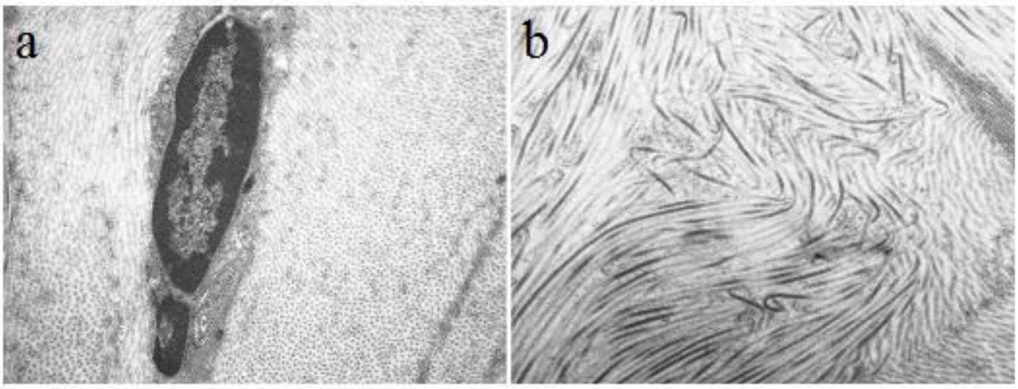

Figure 4. Electron microscopy pictures of cornea on day 21 after the start of the experiment *in vivo*. The magnifications are x7000 (a) and x12000 (b).

4. Conclusion

The implantation of the PLA films into the anterior chamber of eye does not induce an inflammatory reaction and does not increase an intraocular pressure. The study showed the possibility of PLA films using as a corneal implant.

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